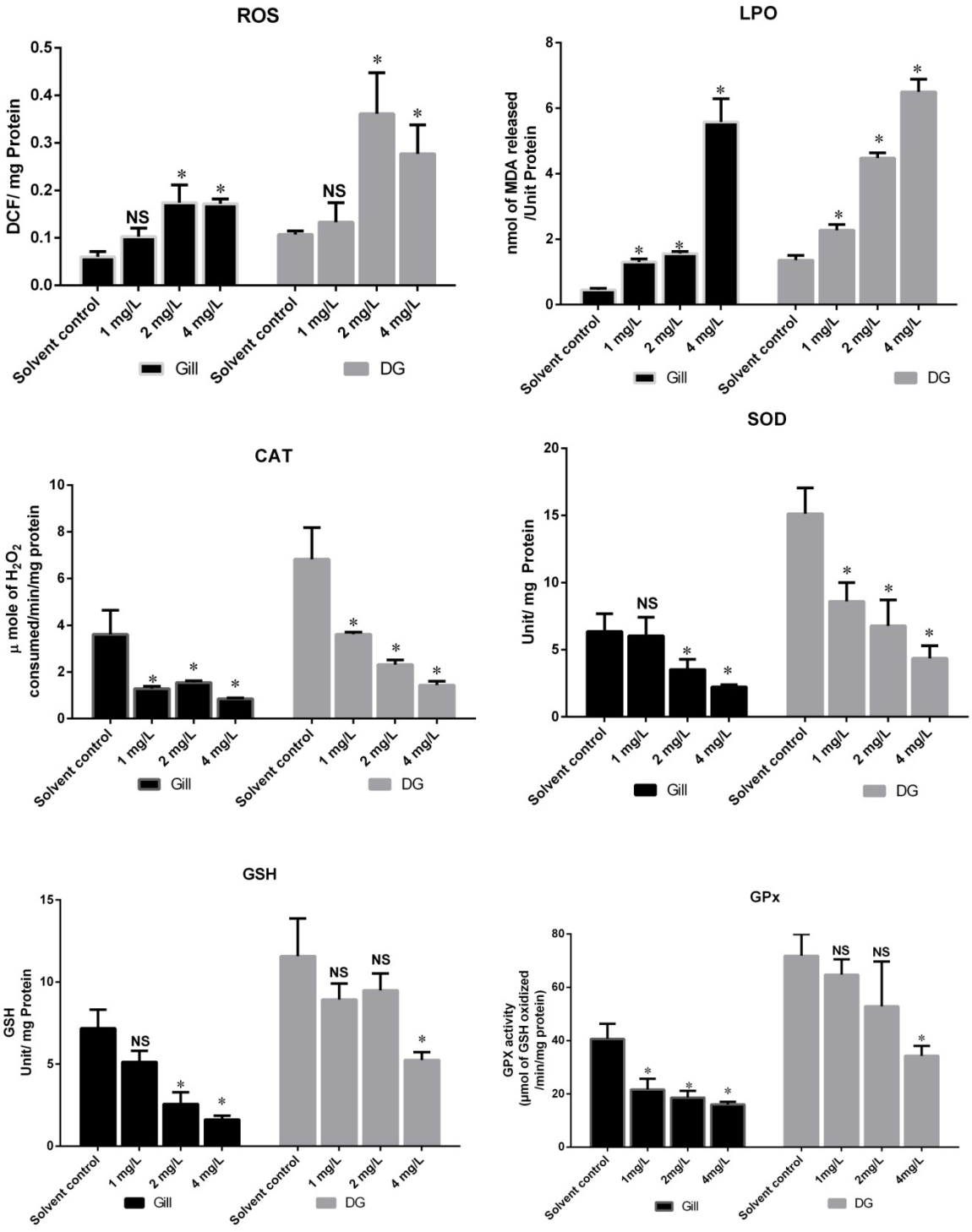
Supplementary figures and tables



Supplementary Fig. 1. Effect of sublethal concentrations of naphthalene on oxidative stress and antioxidant response in *Perna viridis.* Each bar represents mean ± standard error of six determinations using samples from different preparations. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used. The significant difference between control and exposure groups were indicated with asterisks ($P < 0.05$); NS-No significant difference with respective control group.

Table S1 Varimax rotated[a] factor loadings and communality of the biomarker data

Parameters	**VF1**	**VF2**	**Communality**
DG-ROS	0.496	-0.243	0.30
Gill-ROS	0.328	-0.644	0.52
DG-LPO	**0.815**	-0.539	0.96
Gill-LPO	**0.779**	-0.377	0.75
DG-CAT	-0.436	0.606	0.56
Gill-CAT	-0.13	**0.783**	0.63
DG-SOD	-0.338	0.727	0.64
Gill-SOD	-0.609	0.214	0.42
DG-GPx	-0.341	0.428	0.30
Gill-GPX	-0.4	0.536	0.45
DG-GSH	-0.326	0.51	0.37
Gill-GSH	-0.318	**0.816**	0.77
DG-GST	0.671	-0.358	0.58
Gill-GST	0.6	-0.388	0.51
DG-DNA	-0.548	**0.791**	0.93
Gill-DNA	-0.171	**0.873**	0.79
DG-CPR	**0.796**	-0.495	0.88
Gill-CPR	**0.847**	-0.483	0.95
DG-CPO	**0.86**	-0.317	0.84
Gill-CPO	**0.841**	-0.051	0.71
Eigen values	11.20	1.64	
% of Variance	56.01	8.215	
Cumulative %	56.01	64.23	
KMO sampling adequacy	0.638		
Extraction Method: Principal Component Analysis Rotation Method: Varimax with Kaiser Normalization Bold values indicate moderate to strong loadings [a]Rotation converged in 3 iterations			

Supplementary information

Methods for Oxidative stress and Antioxidant assay

ROS measurement

ROS was measured with slight modifications to the method of 2-7-dichlor-odi hydrofluorescein diacetate(DCFH-DA) transformation described by Driver et al. (2000). Homogenates (20 µL), 100 µL physiological saline and 5 µL of DCFH-DA were added to each well and the plates were incubated at 37 ^{o}C for 30 min. Background fluorescence (conversion of

DCFH to dichloro di hydrofluorescine (DCF) in the absence of sample) was corrected by the inclusion of parallel blanks. Conversion of DCFH to the fluorescent product DCF was measured using a TECAN spectrophotometer with excitation at 485 nm and emission at 530 nm. The rate of conversion is directly proportional to the concentration of ROS.

Catalase activity

The activity of catalase (CAT) was measured using Sinha's method (1972). When dichromate in acetic acid was heated in the presence of hydrogen per oxide (H_2O_2), it was converted to chromic acetate, with perchromic acid as an unstable intermediate. At 570 nm, chromatic acetate was measured calorimetrically. The reaction was allowed to run for various amounts of time before being stopped by adding a dichromate:acetic acid combination. The residual H_2O_2 was estimated by colorimetrically measuring the chromic acetate. The activity was measured in mol of H_2O_2 consumed per minute per mg of protein.

Superoxide dismutase activity

The activity of superoxide dismutase (SOD) was determined using the Marklund and Marklund method, (1974) which evaluated the degree of inhibition of auto-oxidation of pyrogallol at an alkaline pH. The quantity of enzyme (per protein milligramme) that suppresses the oxidation process by 50% of its maximum inhibition is defined as one unit of SOD activity. Using phosphate buffered saline buffer, 0.75 mM Nitro Blue Tetrazolium (NBT) solution and 30 mM Xanthine stock solution were prepared. Similarly, just before the analysis, 0.1 M EDTA solutions were prepared and diluted into a 3mM solution. Dilution of prepared compounds was carried out shortly before to the testing. The reaction was then stopped by adding 6 mM CuCl2 solution to a 0.15 percent Bovine Serum Albumin (BSA) (W/V) working solution. Each test required 6 mU of (Xanthine oxidase) XOD (ice cold). SOD activity was determined from the prepared samples.

Reduced Glutathione (GSH)

Reduced glutathione levels were estimated by the method of Moron *et al.,* (1979) by reading the optical density of the yellow substance formed when 5, 5'-dithio-2-nitrobenzoic acid (DTNB) is reduced by glutathione at 412 nm. Up to 2 mM, the reaction rate is proportional to the glutathione concentration. The test determines the quantity of glutathione in the biological sample using a standard curve of reduced glutathione. For 5 minutes, the plate reader was set at 412 nm with kinetic readings at 1 minute intervals. Every test was performed in duplicate. The first 2 wells contain only 10 µl of the 5% 5-sulfosalicylic acid solution as a reagent blank. In

each well of the plate, ten µl of glutathione standard solutions were added. Variable quantities of the unknown sample were added in duplicate to separate wells, followed by 150µl of the working mixture added to each well with a multichannel pipette. The samples were then incubated for 5 minutes at room temperature before being treated with 50 µl of diluted NADPH solution through a multichannel pipette. The absorbance was then measured with a plate reader.

Glutathione peroxidase activity

Gluatathione peroxidase (GPx) activity was determined by measuring the quantity of reduced glutathione (GSH) consumed in the reaction mixture using the Rotruck *et al.,* method (1973). In a brief, the Glutathione peroxidase assay buffer was pipetted into the quartz cuvette. 50 µl of the NADPH assay reagent was poured to the cuvette, followed by 10–50 µl of sample and thoroughly mixed. For all of the samples examined, the total volume in the cuvette was kept constant at 1 ml. The reaction was initiated by adding 30mM tetra-butyl hydroperoxide solution. A kinetic programme (15 seconds) was used to measure the absorbance at 340 nm. Interval: 10 seconds; number of spectrophotometer readings: 6

Glutathione *S* transferase

The glutathione S transferase activity of the fraction obtained with the substrate 1-chloro-2,4-dinitrobenzene (CDNB) was evaluated spectrophotometrically at 37°C after conjugation of the acceptor substrate with glutathione as reported in Habig *et al.,* (1974) and Jakoby (1978). The reaction was monitored by observing at the conjugation of CDNB with reduced glutathione (GSH). The rise in absorbance at 340nm was used to accomplish this. Every minute at 25°C, one unit of enzyme may conjugate 10.0 nmol of CDNB with reduced glutathione. Before measuring, the powdered CDNB was thawed at room temperature to avoid solids condensation. 100 mM CDNB was dissolved in ethanol, aliquoted, and kept at -20°C in microcentrifuge tubes. In the same way, 100 mM reduced Glutathione was made and stored. One millilitre of assay mixture was produced for each test. Before reading the absorbance, 980 µl of TBS (Tris buffered solution) pH 6.5, 10 µl of 100 mM CDNB, and 10 µl of 100 mM Glutathione were added and mixed well. The solution was hazy at first, but after mixing, it became transparent. For the blank solution, 900 µl of enzyme cocktail and 100 µl of TBS were mixed together. Instead of buffer, 100 µl of sample and 900 µl of cocktail were utilised for sample analysis. Absorbance was measured at 340 nm for five minutes, and the findings are reported as conjugate formation per minute per milligramme of protein.